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Deciphering the Effect of *Tetrapleura tetraptera* Saponins on Carbohydrate Metabolizing Enzymes in STZ-Diabetic Wistar Rats: A Gene Expression Study

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ABSTRACT: *Tetrapleura tetraptera* (Taub), widely utilized in traditional African medicine to manage various ailments, including diabetes, is a rich source of bioactive and therapeutic compounds, especially saponins. This study assessed saponins from *T. tetraptera* root bark for modulating carbohydrate-metabolizing enzymes in STZ-induced diabetic male Wistar rats. The genes evaluated were glucokinase, phosphofructokinase-1 (PFK-1), pyruvate kinase, glucose-6-phosphatase (G-6-Pase), fructose-1,6-bisphosphatase (F-1,6-BPase), fructose-2,6-bisphosphatase (F-2,6-BPase), phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphate dehydrogenase (G-6-PDH). Forty-two (42) male Wistar rats were allocated into six (6) groups of seven (7) rats each. Diabetic treated rats (Group 3 - positive control) were administered metformin at 100 mg/kg, while Groups 4, 5, and 6 received *T. tetraptera* saponins (TTS) at 10, 20, and 40 mg/kg, respectively, via oral gavage for 12 weeks. The untreated group (Group 2 - negative control) was investigated simultaneously and compared to the normal control (Group 1). The study demonstrates the hypoglycemic potential of TTS in promoting glucose utilization via major carbohydrate pathways through the upregulation of key glycolytic genes (glucokinase, PFK-1) and pentose phosphate (G-6-PDH) pathway genes, along with the downregulation of key gluconeogenic genes (F-1,6-BPase, PEPCK) in STZ-induced diabetic rats. These findings suggest that TTS may modulate hyperglycaemia by enhancing glucose utilization and inhibiting gluconeogenesis.

Keywords: *Tetrapleura tetraptera*, saponins, Diabetes, Gene expression, Carbohydrate metabolism

Introduction

Elevation of blood glucose above the normal threshold depicts diabetes mellitus (DM) (Guzman-Vilca and Carrillo-Larco, 2025). Effective management of diabetes mellitus integrates lifestyle modifications, including physical activity and dietary regulation, alongside pharmacotherapy. Insulin remains essential for type 1 diabetes, whereas type 2 diabetes is typically managed with oral hypoglycemic drugs (Gupta *et al.,* 2021). Although oral hypoglycemic medications are effective in controlling blood glucose levels, their high cost and potential adverse effects, including gastrointestinal problems, hepatic impairment, and skin rashes, pose challenges for long-term management (Arun and Vettath, 2024). Due to these drawbacks, a lot of patients have resorted to the use of herbal medicine as an alternative therapeutic approach for the treatment of diabetes mellitus. This is because these herbal medicines are readily available, cost-effective, believed to have little or no side effects, and ultimately possess multiple therapeutic benefits (Lema *et al.,* 2024).

Tetrapleura tetraptera is one of the widely used antidiabetic plants in Nigeria (Eguavoen and Omonkhua, 2025). *T. tetraptera* is a flowering medicinal plant belonging to the Leguminosae family. This deciduous tree, indigenous to West Africa, is traditionally called "Prekese" in Twi and "Aridan" in Yoruba, reflecting its cultural significance in Ghana and Nigeria (Mensah *et al.,* 2024). *T. tetraptera* has been reported to be a priceless source of bioactive compounds, especially saponins, a signifier of its very high frothing properties

(Omonkhua *et al.,* 2014). Several studies have demonstrated that this plant exhibits notable antidiabetic, antioxidant, and antihyperlipidemic activities in streptozotocin (STZ)-induced diabetic rats (Omonkhua *et al.,* 2014; Ogunlakin and Sonibare, 2024). Targeting carbohydrate-metabolizing enzymes with medicinal plants has emerged as a promising strategy for diabetes management (Nguyen *et al.,* 2025). Important mechanisms of action function via the regulation of hexokinase, fructose 1,6-diphosphatase, alpha amylase, alpha glucosidase etc. (Tchamgoue *et al.,* 2020). Although *T. tetraptera* is known to regulate alpha amylase (Irondi *et al.,* 2013), no study has been done to ascertain the potential of the saponins from *T. tetraptera* to regulate glycolytic, gluconeogenic, and pentose phosphate pathway enzymes in experimental diabetes.

While we have previously demonstrated the ability of *T. tetraptera* saponins (TTS) to lower blood glucose in STZ-diabetic Wistar rats, with concomitant increase in insulin and insulinotropic genes (Eguavoen and Omonkhua, 2025), this study was designed to investigate the *in vivo* potential of TTS on carbohydrate metabolizing enzymes, namely: glucokinase, phosphofructokinase-1 (PFK-1), pyruvate kinase (PK), glucose-6-phosphatase (G-6-Pase), fructose 1,6-bisphosphatase (F-1,6-BPase), fructose-2,6-bisphosphatase (F-2,6-BPase), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphate dehydrogenase (G-6-PDH) in streptozotocin (STZ)-induced diabetic male Wistar rats using gene expression techniques.

Materials and methods

Plant collection, identification, and authentication: The root bark of the plant material (*Tetrapleura tetraptera*) was obtained from Akungba-Akoko, Ondo State, South-Western Nigeria. The plant sample was authenticated at the Department of Microbiology and Botany, University of Ibadan, Nigeria (voucher number UIH22320). The plant material was thoroughly washed under running tap water, after which it was shade-dried and pulverized into very smooth particles.

Extraction of saponins: Saponins were extracted from the root bark of *T. tetraptera* adopting a technique adapted from Hostettmann *et al.,* (1991). The pulverized plant material was extracted with methanol for 72 hours, filtered, and the filtrate was concentrated using a rotatory evaporator at 40°C, followed by freeze-drying. The crude extract was defatted by partitioning with n-hexane and water (1:2 v/v), after which the aqueous layer was collected and freeze-dried. The aqueous extract was further fractionated with ethyl acetate and butanol (1:2 v/v), and the butanol fraction containing the saponins was concentrated using a rotatory evaporator at 40°C, freeze-dried, and stored in an airtight container at 4°C until use.

Animals and experimental protocol: Forty-two (42) adult male Wistar rats, averaging 120 g in weight, were obtained from the Faculty of Life Sciences, University of Benin, Edo State, Nigeria. The animals were housed in a well-ventilated room at the Department of Anatomy, University of Benin, under a 12-h light/12-h dark cycle. The animals were fed twice daily with standard pelleted feed and were given clean water *ad libitum*. Ethical approval for this study was obtained from the Faculty of Pharmacy Ethical Committee, University of Benin, with reference code, EC/FP/020/19.

Induction of diabetes and experimental grouping: Diabetes was induced by intraperitoneal injection of freshly prepared STZ (65 mg/kg buffered at pH 4.5 by 0.1 M citrate) to overnight fasted rats in groups 2 to 6. Seven (7) days following induction, animals with fasting blood sugar ≥ 180 mg/dl were confirmed diabetic and used for the experiment. Animals (normal and STZ-induced diabetic rats) were grouped into six (6) with seven (7) rats each: (1) normal control (untreated normal rats), (2) diabetic control (untreated diabetic rats), (3) positive control (diabetic rats treated with metformin), (4) diabetic rats treated with 10, (5) 20, and (6) 40 mg/kg body weight of TTS. Treatment commenced after the establishment of stable diabetes and lasted for 12 weeks.

Preparation and administration of plant saponins: The *T. tetraptera* total saponins (TTS) fractions obtained after freeze-drying were reconstituted appropriately in distilled water and used to treat the rats. Metformin at 100 mg/kg body weight and various TTS dosages were administered orally (by gavage) every day for the duration of the experiment.

RNA isolation and quantification: RNA was isolated from the liver and pancreas using TRIZOL reagent and RNA extraction kit (Zymo Research, USA). The purity of the isolated RNA was determined by the ratio of absorbance at 260 nm and 280 nm. The concentration of the isolated RNA was determined at an absorbance of 260 nm.

cDNA synthesis: polymerase chain reaction (PCR) and amplification of gene of interest: One microgram (1 µg) of the total RNA was used to synthesize cDNA by reverse transcriptase reaction using Proto script II First Strand cDNA Synthesis Kit (Biolabs, New England) according to the Manufacturer's protocol in a three-step reaction condition: RNA was denatured at 65 °C for 5 mins to remove secondary structures. This was followed by the synthesis of complementary DNA (cDNA) by reverse transcriptase at 42 °C for 1 h. The reaction was subsequently terminated at 80 °C for 5 min to inactivate reverse transcriptase enzyme.

Gene amplification by polymerase chain reaction: Amplification of genes of interest was done by polymerase chain reaction (PCR) as previously described (Elekofehinti *et al.*, 2018). PCR was performed using Master Mix reagent kit (Thermo Scientific, USA) and appropriate primers (Table 1) for desired amplifications in a Multigene Labnet International machine. The amplified genes were separated on 1 % agarose gel electrophoresis in a 1 × Tris-borate-EDTA (TBE) buffer, and the bands were quantified with "ImageJ" software. The GAPDH gene was used to normalize the relative expression level of the respective gene.

Table 1: List of designed, optimized, and synthesized primers specific for each gene

Gene	**Forward primer sequence (**5' – 3'**)**	**Reverse primer sequence (**5' – 3'**)**
Glucokinase (GK)	GTGTACAAGCTGCACCCGA	CAGCATGCAAGCCTTCTTG
PFK-1	CCAGCACTAAGGGGAACTCA	AGTGAGGGAGTGGTGATGTG
Pyruvate kinase	GGAACCCCGTTGACTACTCA	TATGTTTCTCACCGGCCAGT
Glucose-6-phosphatase	GGACTCATCACTGCTTCCCT	AATCCTGACTCTCCCCTTGC
Fructose-1,6-biphosphatase	TGACCCTGCCATCAATGAGT	ATGTCTTCATTCCCCGTCGT
Fructose-2,6-bisphosphatase	GAGATAGAAGACGGACGGGG	CATTTCTTCCCTGCCCTTCG
PEPCK	CAGGATCGAAAGCAAGACGG	ACATAGGGCGAGTCTGTCAG
Glu-6-phosphate dehydrogenase	GCTGGAACCGCATCATAGTG	GTTGGCAAATCTCAGCACCA
GAPDH	AGACAGCCGCATCTTCTTGT	CTTGCCGTGGGTAGAGTCAT

Statistical analysis: Gene expression analysis was done using the ImageJ software. This software helped to estimate densitometrically the thickness of the bands from agarose gel electrophoresis, while Graphpad Prism 8.0.1 (San Diego, California, USA) was used to plot the graph.

Results

Effect of Tetrapleura tetraptera saponins on glucokinase (GK) expression in the liver and pancreas: The effect of TTS on hepatic expression of glucokinase is shown in Figure 1A. In comparison with normal control, there was a significant up-regulation of glucokinase expression in diabetic control. Oral administration of metformin and TTS (10 mg/kg, 20 mg/kg and 40 mg/kg) further significantly up-regulated the hepatic expression of glucokinase relative to diabetic control especially at 10 mg/kg body weight. In Figure 1B, there was significant up-regulation of pancreatic glucokinase expression in the diabetic control group relative to the normal control, metformin, and TTS 40 mg/kg groups. However, a significant up-regulation of the expression of glucokinase was observed after oral administration of TTS at 10 and 20 mg/kg treated group when compared with diabetic control group, with the TTS 20 mg/kg body weight treated group having the highest expression of pancreatic glucokinase enzyme.

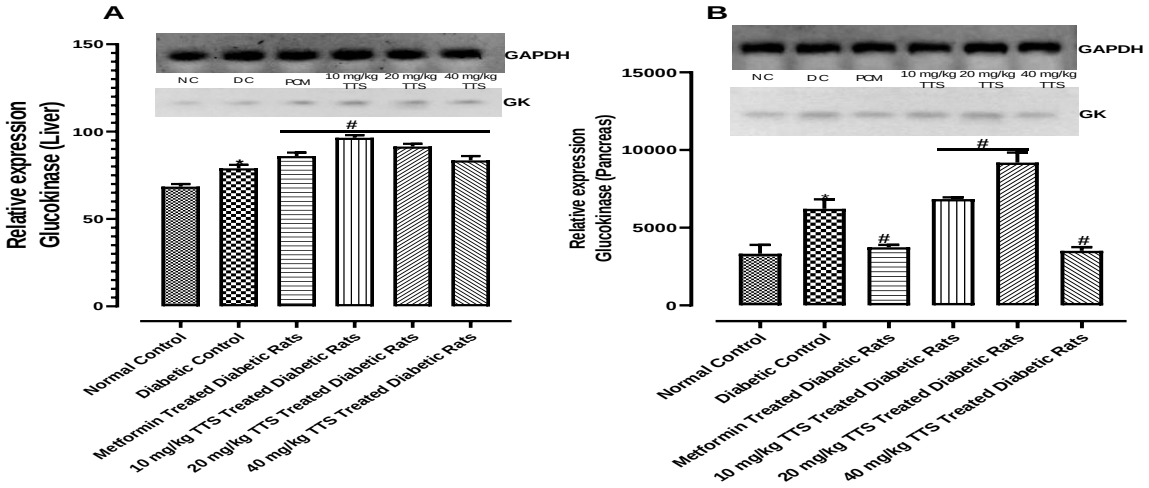

Figure 1: Effect of *Tetrapleura tetraptera* Saponins on glucokinase (GK) expression in the liver (A) and Pancreas (B) of Streptozotocin (STZ) Induced Diabetic Wistar rats. *Represents $p < 0.05$ to Normal Control and # Represent $p < 0.05$ to Diabetic Control. NC= Normal Control, DC= Diabetic Control, PCM= Positive Control (Metformin), TTS= *Tetrapleura tetraptera* saponins.

*Effect of Tetrapleura tetraptera saponins on phosphofructokinase-1 (PFK-1) and pyruvate kinase (PK) expression in the liver***:** The effect of TTS on the gene expression of phosphofructokinase-1 is shown in Figure 2A. Phosphofructokinase-1 expression was significantly up-regulated in diabetic control relative to normal control. While it was demonstrated that oral administration of 10 mg/kg of TTS significantly up-regulated the expression of phosphofructokinase-1 in comparison with diabetic control, administration of 20 mg/kg and 40 mg/kg of TTS down-regulated the expression of phosphofructokinase-1 in comparison with diabetic control. But there was no significant difference between metformin and diabetic control. TTS at 10 mg/kg increased the expression of phosphofructokinase-1 the most.

As shown in Figure 2B, there was significant up-regulation of pyruvate kinase expression in the diabetic control group relative to the normal control group. A significant down-regulation of the expression of pyruvate kinase was observed after oral administration of metformin and TTS (10 mg/kg, 20 mg/kg and 40 mg/kg) when compared with diabetic control.

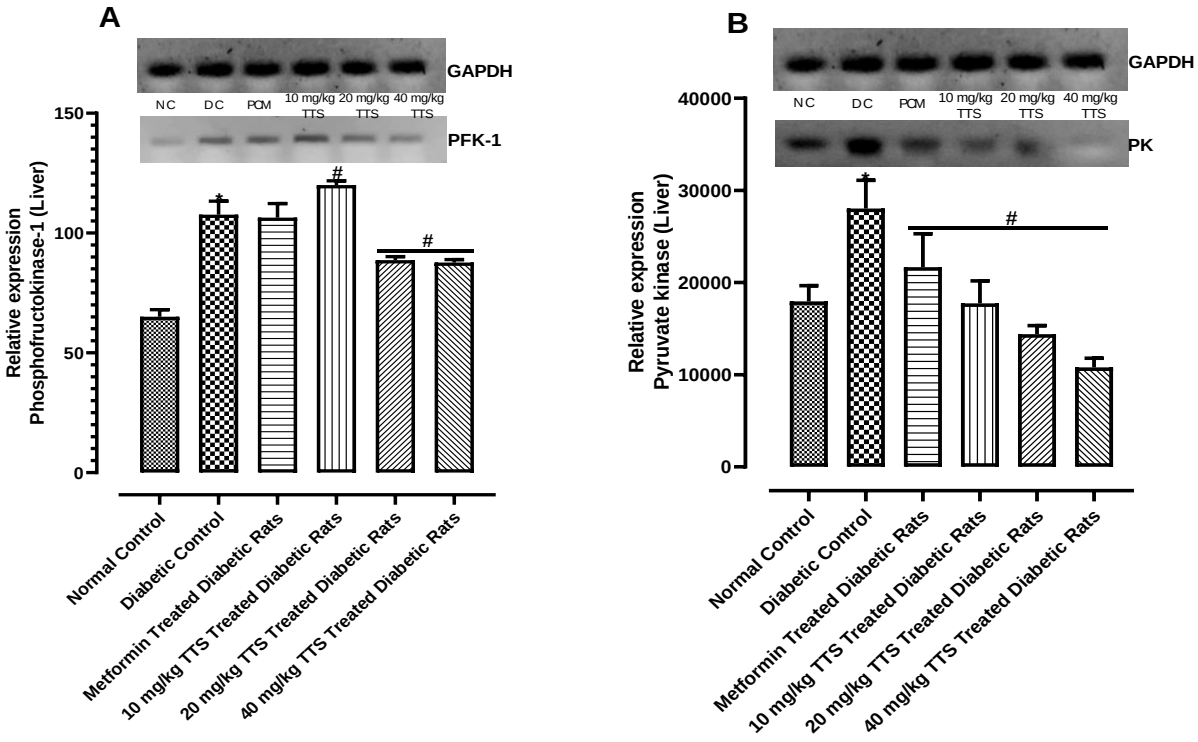

Figure 2: Effect of *Tetrapleura tetraptera* Saponins on Phosphofructokinase-1 (PFK-1) and Pyruvate Kinase (PK) Expression in the Liver of Streptozotocin (STZ) Induced Diabetic Wistar rats. *Represents $p < 0.05$ to Normal Control and # Represent $p < 0.05$ to Diabetic Control. NC= Normal Control, DC= Diabetic Control, PCM= Positive Control (Metformin), TTS= *Tetrapleura tetraptera* Saponins.

*Effect of Tetrapleura tetraptera saponins on glucose-6-Phosphatase (G-6-Pase) and fructose-1,6-bisphosphatase (F-1,6-Pase) expression in the liver***:** The effect of TTS on the relative expression of glucose-6-phosphatase is shown in Figure 3A. Compared to the normal control, there was a significant down-regulation of glucose-6-phosphatase expression in the diabetic control group. Oral administration of TTS (10 mg/kg, 20 mg/kg, and 40 mg/kg) significantly up-regulated the expression of glucose-6-phosphatase relative to diabetic control.

Figure 3B illustrates the effect of TTS and metformin on the expression of fructose-1,6-bisphosphatase in diabetic rats. Compared to normal control, there was a significant overexpression of fructose-1,6-bisphosphatase in the diabetic control group. In comparison to the diabetic control, there was a significant ($p < 0.05$) upregulation of fructose-1,6-bisphosphatase expression in the group administered 20 mg/kg TTS, while there was significant downregulation of fructose-1,6-bisphosphatase expression in the group treated with 10 mg/kg TTS.

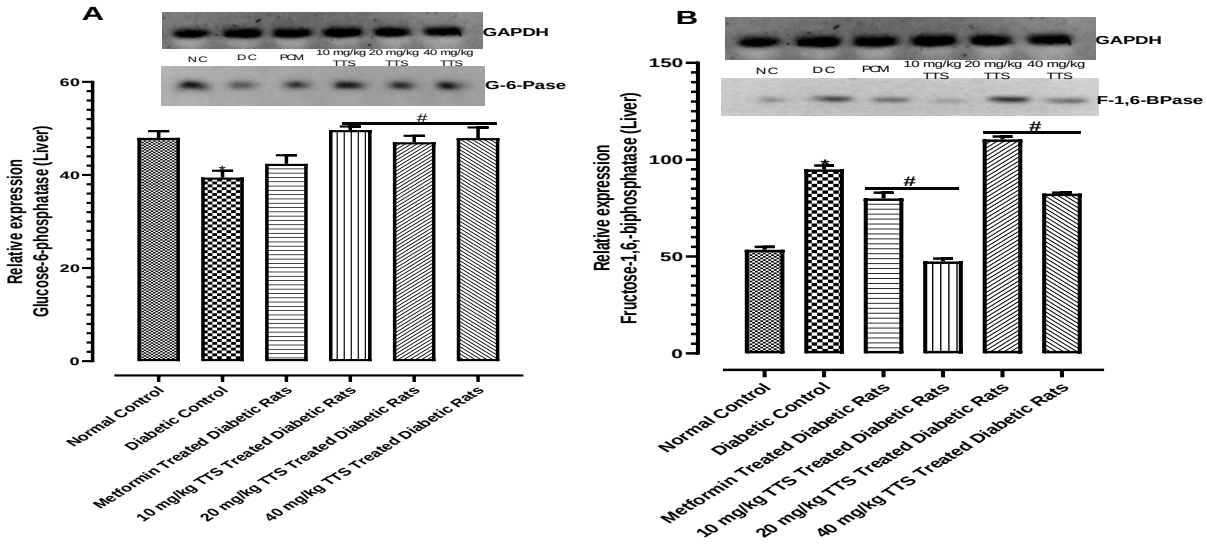

Figure 3: Effect of *Tetrapleura tetraptera* Saponins on Glucose-6-Phosphatase (G-6-Pase) (A) and Fructose-1,6-Bisphosphatase (F-1,6-Pase) (B) Expression in the Liver of Streptozotocin (STZ) Induced Diabetic Wistar rats. *Represents $p < 0.05$ to Normal Control and # Represent $p < 0.05$ to Diabetic Control. NC= Normal Control, DC= Diabetic Control, PCM= Positive Control (Metformin), TTS= *Tetrapleura tetraptera* saponins.

Effect of Tetrapleura tetraptera saponins on fructose-2,6-bisphosphatase (F-2,6-Pase) and Phosphoenolpyruvate Carboxykinase (PEPCK) expression in the liver: The effect of TTS and metformin on the relative expression of fructose-2,6-bisphosphatase is shown in Figure 4A. In comparison with normal control, there was a significant upregulation of fructose-2,6-bisphosphatase expression in diabetic control. Oral administration of metformin and TTS (10, 20, and 40 mg/kg) significantly upregulated the expression of fructose-2,6-bisphosphatase relative to diabetic control.

There was significant upregulation of PEPCK expression in diabetic control relative to normal control (Figure 4B). Amongst the treatment groups, barring TTS at 10 mg/kg body weight, it was observed that oral administration of metformin and TTS at 20 and 40 mg/kg, especially TTS at 40 mg/kg body weight, significantly down-regulated the expression of PEPCK when compared with the diabetic control group to inhibit the expression of the gluconeogenic enzyme PEPCK.

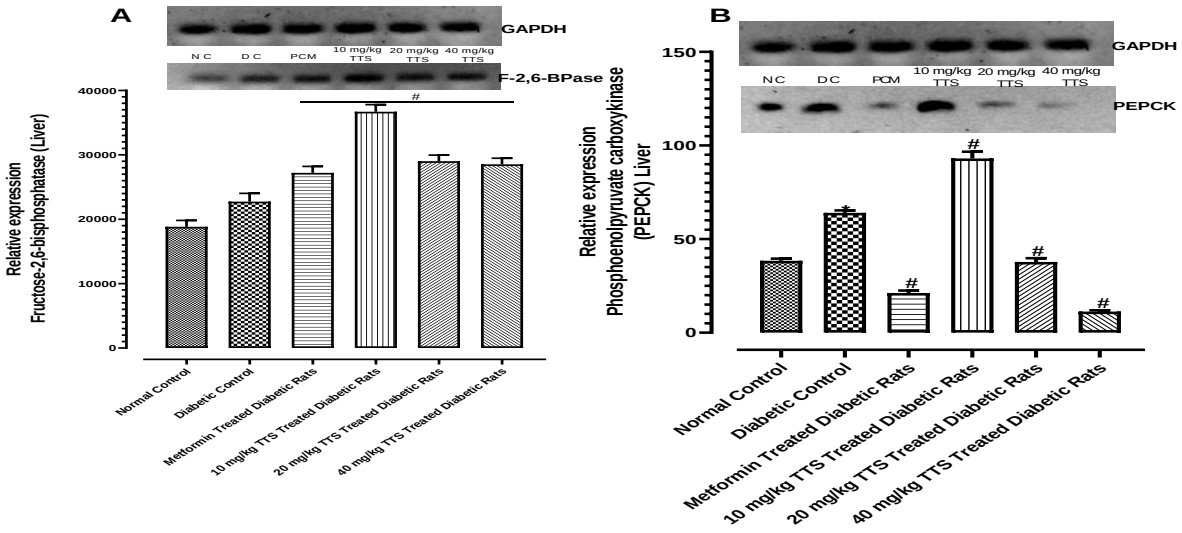

Figure 4: Effect of *Tetrapleura tetraptera* Saponins on Fructose-2,6-Bisphosphatase (F-2,6-Pase) (A) and Phosphoenolpyruvate Carboxykinase (PEPCK) (B) Expression in the Liver of Streptozotocin (STZ) Induced Diabetic Wistar rats. *Represents $p < 0.05$ to Normal Control and # Represent $p < 0.05$ to Diabetic Control. NC= Normal Control, DC= Diabetic Control, PCM= Positive Control (Metformin), TTS= *Tetrapleura tetraptera* Saponins.

Effect of Tetrapleura tetraptera saponins on glucose-6-phosphate dehydrogenase (G-6-PDH) expression in the liver: As shown in Figure 5, there was significant upregulation of glucose-6-phosphate dehydrogenase expression in diabetic control group relative to normal control group. A significant upregulation of glucose-6-phosphate dehydrogenase expression was observed after oral administration of TTS (10, 20, and 40 mg/kg) in a dose-dependent manner when compared with the diabetic control group. There was no significant difference between the metformin-treated group and the diabetic control group in the expression of glucose-6-phosphate dehydrogenase.

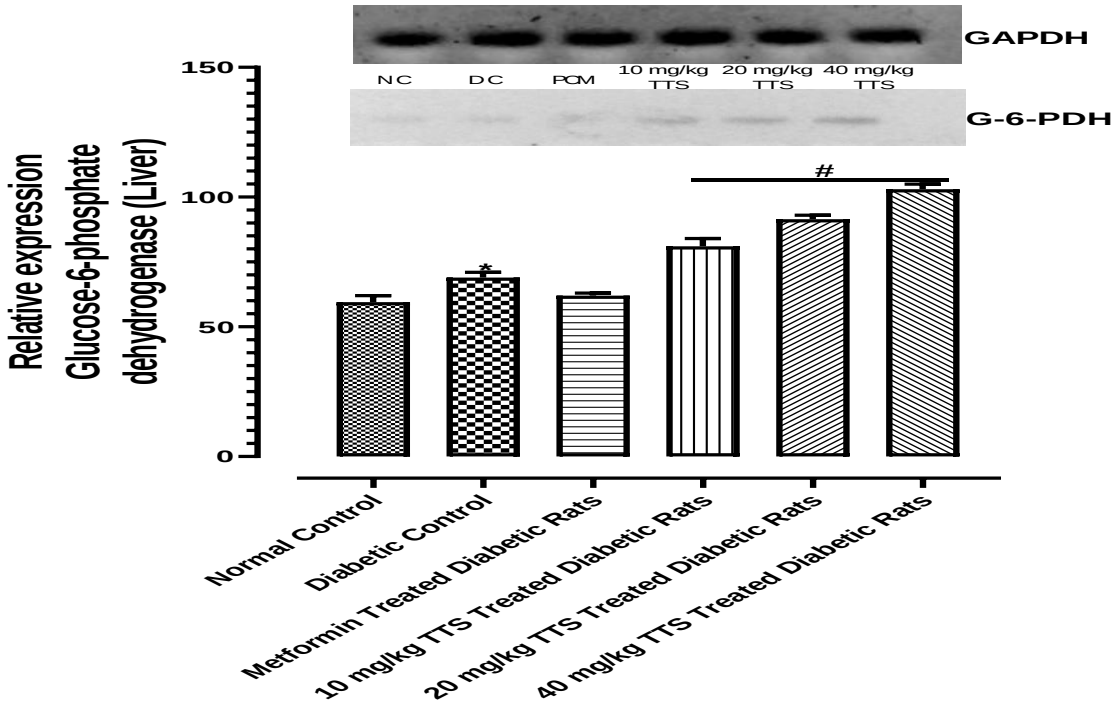

Figure 5: Effect of *Tetrapleura tetraptera* saponins on Glucose-6-Phosphate Dehydrogenase (G-6-PDH) Expression in the Liver of Streptozotocin (STZ) Induced Diabetic Wistar rats. *Represents $p < 0.05$ to Normal Control and # Represent $p < 0.05$ to Diabetic Control. NC= Normal Control, DC= Diabetic Control, PCM= Positive Control (Metformin), TTS= *Tetrapleura tetraptera* saponins.

Discussion

Medicinal plants are a rich source of diverse bioactive compounds, including saponins, flavonoids, phenols, and alkaloids. Even with modern advancements in drug discovery, they continue to play a central role in healthcare for a substantial portion of the global population (Abdallah *et al.,* 2023). *Tetrapleura tetraptera* Taub. possesses hypoglycaemic, antioxidant, and antihyperlipidaemic properties in streptozotocin (STZ)-induced diabetes (Omonkhua *et al.,* 2014).

Our previous studies also show that *T. tetraptera* saponins (TTS) lower blood glucose via increase in serum insulin and insulinotropic genes (Eguaveon and Omonkhua, 2025). Carbohydrate-metabolizing enzymes, including those involved in glycolysis and gluconeogenesis, are critical for maintaining glucose homeostasis and have emerged as promising targets in the management of diabetes (Tchamgoue *et al.,* 2020). Glucokinase plays a pivotal role in glycolysis and is now considered a potential therapeutic target for antihyperglycemic agents in diabetes treatment (Sharma *et al.,* 2022). Alterations in the glucokinase (GK) gene can affect the enzyme's affinity for glucose, thereby perturbing normal glucose homeostasis and contributing to abnormal blood glucose (Jiang *et al.,* 2021).

From this study, the effect of TTS on liver glucokinase showed up-regulation amongst all groups (Figure 1A). But the expression was highest in the 10 mg/kg TTS treated group. Pancreatic glucokinase expression (Figure 1B) was also up-regulated in the groups administered 10 mg/kg TTS and 20 mg/kg when compared to the diabetic control group. This hypoglycemic effect of TTS shows that it can facilitate glucose uptake via the stimulation of the glucokinase gene which ultimately leads to the release of insulin (which further enhances glucose uptake).

The conversion of glucose to glucose-6-phosphate upon cellular uptake is a key step in glycolysis, providing the substrate for subsequent reactions that generate energy for cellular processes (Salih *et al.,* 2022). As a central glycolytic intermediate, glucose-6-phosphate can be directed toward glycogen synthesis through glycogenesis (Salih *et al.,* 2022) or utilized in the pentose phosphate pathway to produce nucleotides and reducing equivalents (NADPH), all of which are needed for cellular function (Rajas *et al.,* 2019).

This mechanism shows that TTS has the potential to be a hypoglycemic agent. The ethnopharmacological perspectives of glucokinase activators in the treatment of diabetes mellitus was conducted in a study by Sharma *et al.* (2022), while Mata-Torres *et al.* (2021) also reported on the approaches to decrease hyperglycemia by targeting impaired hepatic glucose homeostasis using medicinal plants.

The relative expression of phosphofructokinase-1 gene, the enzyme that regulates the rate limiting step (step 3) of glycolysis, was also shown from our study (Figure 2A) to be up-regulated by TTS. Although the significant upregulation of the diabetic control's PFK-1 gene is puzzling, the 10 mg/kg TTS-treated group induced the highest expression of PFK-1 gene. The upregulation of PFK-1 by TTS, particularly at a dosage of 10 mg/kg TTS, suggests efficient glucose utilization by the cells and indicates a promising pharmacological strategy for treating and regulating glucose homeostasis, which is skewed in diabetes (Elekofehinti *et al.,* 2014).

Surprisingly, pyruvate kinase was significantly down-regulated (Figure 2B) in all test groups compared to the diabetic control group. The reason for this is unclear, however it implies that while TTS does not modulate this enzyme directly, the influx of intermediates from PFK-1 will favour this reaction. This does not preclude the anti-diabetic potential of TTS in up-regulating glycolytic enzymes, as we have already observed these potentials in steps 1 and 3 (Figure 1A, 1B, and 2A), particularly in step 3, which is the most critical step in regulating glycolysis.

Glucose-6-phosphatase plays a key role in gluconeogenesis during hypoglycemia, converting glucose-6-phosphate into free glucose, which can then be exported into the bloodstream to maintain glucose homeostasis (Xia *et al.,* 2025). When blood glucose levels are high, this pathway is inhibited. Down-regulation of this enzyme in the untreated diabetic group is curious, as the diabetic state normally favours gluconeogenesis. This observation may be attributed to severe pancreatic β-cell damage caused by streptozotocin, which induces oxidative stress and disrupts metabolic regulation, thereby altering hepatic gene expression (Lao-ong *et al.,* 2012). The reduction, relative to normal control of glucose 6-phosphatase in the metformin group, implies inhibition of gluconeogenesis, which is favourable. However, the increase in glucose 6-phosphatase in all TTS groups relative to diabetic control implies that this extract was not able to inhibit gluconeogenesis at this point. A different scenario was observed with fructose-1,6-bisphosphatase gene expression by TTS. With the exception of the 10 mg/kg TTS group, all other groups up-regulated this enzyme relative to normal control, again showing the therapeutic benefit of TTS at this dose. This therapeutic potential of saponins in various medicinal plants has been widely reported in ameliorating diabetic complications by inhibiting gluconeogenesis (El Barky *et al.,* 2017; Shehadeh *et al.,* 2021).

Importantly, this step in gluconeogenesis, as with glycolysis, is the rate limiting step and the most highly regulated step. The excellent action of 10 mg/kg TTS treated group on fructose-1,6-bisphosphatase, showed its ability to inhibit gluconeogenesis. Recall from our result in Figure 1A, that 10 mg/kg TTS had the best activity in increasing the expression of PFK-1. So, in the rate limiting steps of glycolytic and gluconeogenesis, 10 mg/kg TTS on one hand is favouring glycolysis by increasing the expression of PFK-1 and also stopping gluconeogenesis by inhibiting fructose-1,6-bisphosphatase enzyme activity.

From the result in Figure 4A, there was a significant up-regulation of fructose-2,6-bisphosphatase (F-2,6-BPase) in all treatment groups, and the untreated diabetic group in contrast with the control group. Fructose-2,6-bisphosphatase, part of the bifunctional enzyme PFK-2/FBPase-2, plays a crucial role in regulating glycolysis and gluconeogenesis (Tan *et al.,* 2026). It catalyzes the conversion of fructose-2,6-bisphosphate (Fru-2,6-P2) to fructose-6-phosphate, thus decreasing the concentration of Fru-2,6-P2. Fructose-2,6-bisphosphate acts as an allosteric regulator, promoting glycolysis and inhibiting gluconeogenesis (Tan *et al.,* 2026).

This infers that all TTS- and metformin-treated groups may not have good control of fructose-2,6-bisphophatase expression at this point in inhibiting gluconeogenesis; however, relative to the untreated group, TTS and metformin treated groups were able to inhibit gluconeogenesis at other points, key of which is the rate limiting step, by down-regulating fructose-1,6-bisphophatase expression especially TTS 10 mg/kg (Figure 3B). Thus, this could infer that TTS are potentially good therapeutic agents in regulating glucose homeostasis at rate limiting points in glucose metabolism.

The effect of TTS on phosphoenolpyruvate carboxykinase (PEPCK) gene expression from our result (Figure 4B), showed that apart from 10 mg/kg TTS, all treated groups, and metformin, significantly down-regulated the expression of PEPCK genes compared to diabetic control. It is worth noting that 40 mg/kg TTS appeared to have the lowest expression of PEPCK, thereby effectively inhibiting gluconeogenesis. Medicinal plants have been shown to elevate plasma insulin levels, which may suppress key gluconeogenic enzymes, including fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase (Mata-Torres *et al.,* 2021; Cui *et al.,*

2018).
The effect of TTS on glucose-6-phosphate dehydrogenase (G6PD) in the pentose phosphate pathway was also assessed. It is important to note that during high blood glucose levels, the pentose phosphate pathway is very important in glycemic control by converting glucose-6-phosphate (glycolytic intermediate) to reducing equivalent (NADPH) and metabolites for nucleotides biosynthesis (Rajas *et al.*, 2019). The first and rate-limiting enzyme in this pathway is glucose-6-phosphate dehydrogenase (G-6-PDH) (Aydemir and Ulusu, 2023). Deficiency in G-6-PDH has been reported to increase reactive oxygen species as a result of decreased production of NADPH, which can initiate oxidative stress to bring about diabetic complications (Çelik *et al.*, 2022). It was observed from the result of this study that TTS increased the expression of glucose-6-phosphate dehydrogenase in a dose-dependent manner amongst all TTS-treated groups. This effect is another mechanism by which *T. tetraptera* saponins can maintain glucose homeostasis by favouring the pentose phosphate pathway. Up-regulation of this important rate limiting enzyme by TTS is an important source of NADPH production which can be used to synthesize reduced glutathione (GSH) that provides defenses against reactive oxygen species and thus ameliorate oxidative damage in diabetes.

Conclusion

The current findings from this study demonstrate the possible hypoglycemic mechanisms of *T. tetraptera* saponins (TTS) on STZ-induced diabetic Wistar rats. *T. tetraptera* saponins (TTS) demonstrated regulatory effects on carbohydrate metabolizing genes by significantly upregulating major glucose utilizing enzymes (glucokinase, PFK-1) and pentose phosphate (G-6-PDH); while at the same time down-regulating gluconeogenic genes (F-1,6-BPase, PEPCK). These findings suggest that the enhancement of glucose utilization by TTS, especially at 10 mg/kg body weight, may be one of its glucose lowering mechanisms.

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Ethical considerations

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Faculty of Pharmacy Ethical Committee, University of Benin, with the reference number EC/FP/020/19.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Data availability

All data used is available in the manuscript.

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